the presence of functional BAT, which appeared recruitable by cold acclimation. Molecular analysis confirmed the tissue specific expression of UCP1 in tenrecs (etUCP1). We cloned and stably transfected etUCP1 in HEK293 cells. Isolated mitochondria of etUCP1 HEK293 cells showed inducible proton conductance using palmitate, and GDP-sensitivity, similar to mitochondria containing mouse UCP1. For the search of functional differences, we established bioenergetic measurements in intact HEK293 cells using plate-based respirometry, allowing high-throughput approaches for small molecule modulators. In the initial experiments, we show that a cell-permeable UCP1 activator allows direct specific activation of tenrec and mouse UCP1.

Taken together, we show that *E. telfairi* possesses functional, UCP1-dependent brown adipose tissue, which may facilitate active rewarming from hypothermic states. Substantial evolutionary distance between tenrecs and modern mammals provides a new window to study the evolution of structure-function relationships of UCP1.

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5P11

The breath of a fruit-fly

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The fruit-fly *Drosophila melanogaster* is widely used as a model organism to study human diseases, including those affecting mitochondria. Most mutant flies engineered to mimic mtDNA diseases die at the larval stages, yet a detailed bioenergetic characterization of developmental stage-specific features of Drosophila has never been carried out. We used muscular body-wall Drosophila larvae preparations to study respiration with the sensitive Seahorse[a] technology. This method allows us to study individual larvae and therefore whole tissue bioenergetics *in situ*. Larvae maintained a steady respiratory rate which was inhibited (i) by rotenone and antimycin A, demonstrating its mitochondrial origin and (ii) by 2-iodoacetate, suggesting that respiration is coupled to a high glycolytic flux. Unexpectedly, respiration could not be decreased by the F1FO ATPase inhibitor oligomycin nor increased by the uncoupler FCCP, suggesting that in Drosophila larvae mitochondria are uncoupled. Consistent with a developmental stage-specific uncoupling effect, the respiratory profile of embryonic multilineage Drosophila $S2R^{+}$ cells was instead essentially similar to that of mammalian cells in culture, as basal respiration could be inhibited by oligomycin and then stimulated by FCCP. Sequence homology analysis revealed the existence of four putative uncoupling proteins (UCPs) in Drosophila (UCP4a, UCP4b, UCP4c, and UCP5) that share 60–70% homology with their mammalian counterparts. Whether these are developmentally regulated is not known. We suspect that in the poikilotherm Drosophila uncoupling may be essential for thermogenesis in the pre-pupal stages. We are carrying out silencing of each UCP transcript in whole larvae, as well as genetic ablation of individual UCP genes, to address the potential role of UCP proteins in larval respiration.

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5P12

UCP2 is associated with a high cell proliferative potential and therefore not present in neurons

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Since its discovery 15 years ago the transport function and the involvement of the inner mitochondrial membrane uncoupling protein 2 (UCP2) in diabetes, obesity, arteriosclerosis, neurodegenerative and neuroinflammatory diseases is controversially discussed. Moreover its tissue distribution is still uncertain, especially the presence of the protein in brain. Here, we re-evaluate the UCP2 expression pattern at mRNA and protein level and reveal a strong association of UCP2 with cells and tissues of the immune system, in particular with T-, B-, NK- cells and monocytes. Activation of T-cells leads to a 10-fold increase in UCP2 abundance. The late onset of UCP2 up-regulation in activated T-cells indicates a role of UCP2 in the later events of the immune response like metabolism increase and cell proliferation. We found only UCP4 (1, 2) and not UCP2 in neurons, although we confirmed the presence of UCP2 mRNA in brain. Instead, we detected UCP2 in microglia cells. Together with the reports from other research groups showing the presence of UCP2 in stem and cancer cells, our present results support the idea that UCP2 up-regulation is associated with the proliferative potential of cells.

[1] Smorodchenko A, Rupprecht A, Sarilova I, Ninnemann O, Bräuer AU, Franke K, Schumacher S, Techritz S, Nitsch R, Schuelke M, Pohl EE (2009) Biochim Biophys Acta 1788(10):2309-19.
[2] Smorodchenko A, Rupprecht A, Fuchs J, Gross J, Pohl EE (2011) Mol Cell Neurosci. 47(4):244–53.

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5P13

UCP2 in tumour cells: Analyzing its role in the defence against oxidative stress

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The biochemical activity of the uncoupling proteins (UCPs) is to lower the efficiency of the oxidative phosphorylation presumably by increasing the membrane proton conductance. This mild uncoupling would lead to an increase in respiration that should result in a lower generation of superoxide. This uncoupling activity has been proposed to constitute a mechanism of defence against oxidative stress and, in fact, it has been reported that UCPs are upregulated in physiological situations where there is oxidative stress and, furthermore, that their overexpression reduces ROS damage.

Tumour cells have a high intrinsic level of oxidative stress and, in these cells, UCP2 could play a defensive role [1]. Thus, it has been shown that in colon cancer UCP2 expression is increased and that

this induction appears linked to NF-kB activation and oxidative stress. Increased UCP2 levels have also been associated with resistance to chemo- and radiotherapy in sublines of leukemia and melanoma. Additionally, it has been shown that overexpression of UCP2 in tumour cells reduces ROS levels and apoptosis when treated with antitumour drugs such as doxorubicin or camptothecin.

Two small ligands have been reported to inhibit UCP2: genipin and chromane derivatives. Inhibition of UCP2 in tumour cells by these compounds has been reported to cause oxidative stress and to sensitize the cells against chemotherapeutic agents that cause oxidative stress like arsenic trioxide, doxorubicin or menadione [2,3]. These small molecules may, however, affect other cellular functions and increased ROS levels may not be a direct consequence of UCP2 inhibition. We show how detailed analysis of the effect of these compounds on the energy metabolism and oxidative stress parameters helps to assess their biological actions.

References

[1] G. Baffy, Mitochondrion 10 (2010) 243–252.
[2] R.J. Mailloux, C.N. Adjeitey, M.E. Harper, PLoS One 10 (2010) e13289.
[3] E. Rial, L. Rodríguez-Sánchez, P. Aller, A. Guisado, M.M. González-Barroso, E. Gallardo-Vara, M. Redondo-Horcajo, E. Castellanos, R. Fernández de la Pradilla, A. Viso, Chem. Biol. 18 (2011) 264–274.

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5P14

Functional characterization and regulation of UCP4 expression by adipokinetic hormone in larva and pupa fat body mitochondria from the beetle *Zophobas atratus*

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Uncoupling protein 4 (UCP4) is the member of the large family of mitochondrial anion transporters that uncouple oxidative phoshorylation. The present study demonstrates for the first time, the molecular identification of a partial *Zophobas atratus* UCP4 coding sequence and the functional characterisation of ZaUCP4 in the mitochondria of larval and pupal fat bodies of the beetle. ZaUCP4 shows a high similarity to predicted insect UCP4 isoforms and known mammalian UCP4s, both at the nucleotide and amino acid sequence levels. Bioenergetic studies unequivocally demonstrates UCP4 activity in mitochondria isolated from larvae and pupae fat body. In resting, non-phosphorylating state 4 respiration ZaUCP activity was stimulated by palmitic acid and inhibited by the purine nucleotide GTP. In phosphorylating mitochondria, ZaUCP4 activity decreased the yield of oxidative phosphorylation. ZaUCP4 was immunodetected by using of antibodies raised against human UCP4 as a single 36 kDa band. Because it known that hormones influence an expression of UCPs, we tested adipokinetic hormone (AKH), analogue of mammalian glucagon, which mobilizes lipids and carbohydrates from fat body stores. Besides this energy-mobilizing function, AKHs inhibit fat body lipid and protein synthesis. After AKH injection, we observed the decrease in ZaUCP4 expression at the mRNA and protein levels in both developmental stages of the beetle.

Real time analysis, immunological detection and bioenergetic characteristic indicate consistently for the higher expression of UCP4 *in* the *Z. atratus* larval fat body compared with the pupal fat body, furthermore ZaUCP4 expression is under hormonal control. The different expression pattern and activity of ZaUCP4 during the larval-pupal transformation indicates an important physiological role for UCP4 in insect fat body development and function during insect metamorphosis. Furthermore, the regulation of UCP4 expression by AKH indicates that it may play role in maintaining of metabolic homeostasis in the insect.

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5P15

UCP2 up-regulation during experimental autoimmune encephalomyelitis (EAE) correlates with lymphocyte infiltration

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Experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), is characterized by inflammation and demyelination of the central nervous system (CNS) and associated with severe neurological disability, where reactive oxygen species (ROS) and mitochondrial dysfunction play a pivotal role. Uncoupling proteins (UCPs) belong to the mitochondrial anion transporter family and were proposed to regulate ROS. In particular, UCP2 has been shown to be associated with autoimmune neurodegenerative disease MS and EAE [1]. Our previous results showed that under physiological conditions UCP4 [2; 3] and UCP2 are present in neuronal and microglia cells of CNS respectively. Here we compare the role of UCP2, UCP4 and UCP5 in EAE by study of protein distribution pattern in mouse CNS using RT-PCR and Western blot. Mice were immunized either by myelin oligodendrocyte glycoprotein (MOG) to induce EAE or by CNS-irrelevant protein ovalbumin (OVA) as a control. We found neuronal injury, axonal loss, reactive astro- and microgliosis only in CNS of MOG-EAE but not in that of OVA-immunized mice. The UCP5 was undetectable in both investigated groups, no alteration in UCP4 abundance was observed. In contrast, UCP2 and $CD3^+$ up-regulation was detected in spinal cord (SC) of OVA- and MOG-immunized mice indicating the correlation of UCP2 amount with T-lymphocyte tissue infiltration.

Our results provide evidence that mitochondrial UCP2 rather than UCP4 is involved in course of EAE and support the hypothesis that the protein up-regulation is due to unspecific T-lymphocytes activation leading to cell proliferation and cell invasion into CNS.

References

[1] Vogler, et al., Am. J. Pathol. 168 (2006) 1570–1575.
[2] Smorodchenko, et al., Mol. Cell Neurosci. 47 (4) (2011) 244–253.
[3] Smorodchenko, et al., Biochim. Biophys. Acta 1788 (10) (2009) 2309–2319.

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